Int'l Appl. No.: PCT/AU03/01647
Int'l Filing Date : December 9, 2002

10/538393

JC17 Rec'd PCT/PTO 08 JUN 2005

## AMENDMENTS TO THE CLAIMS

1.      **(Currently Amended)**      A method of generating a population of T-cells specific for an antigen, said method comprising:

isolating a population of substantially mature antigen presenting cells (APC)[[,]]: and

co-incubating the substantially mature APC population with a population of CD4+ T-cells, a population of CD8+ T- cells and a target antigen for a time and under conditions sufficient to generate CD8+ T-cells specific for said antigen.

2.      **(Original)**      The method of Claim 1 wherein the co-incubation comprises simultaneously admixing two or more of the mature APC, the CD4+ T-cells, the CD8+ T- cells and the target antigen.

3.      **(Original)**      The method of Claim 1 wherein the co-incubation comprises the sequential addition of one or more of the mature APC, the CD4+ T-cells, the CD8+ T-cells and the target antigen in any order.

4.      **(Original)**      The method of Claim 1 wherein the mature APC are co-incubated with a cognate reactive antigen to generate a mature APC population expressing the cognate reactive antigen or a T-cell interacting portion thereof (activated APC).

5.      **(Original)**      The method of Claim 4 wherein the activated APC are co-incubated with a population of CD4+ T-cells.

6.      **(Original)**      The method of Claim 5 wherein the activated APC/CD4+ T-cells are co- incubated with target antigen.

7.      **(Original)**      The method of Claim 6 wherein the activated APC/CD4+ T-cells/antigen mixture is co-incubated with CD8+ T-cells.

8.      **(Original)**      The method of Claim 7 wherein cytotoxic T-cells are selected from the CD8+T- cells.

9.      **(Original)**      The method of Claim 5 wherein the CD4+ T-cells are CD4+ CD25- T- cells.

10.      **(Original)**      The method of Claim 1 wherein the APC are dendritic cells (DC).

11.      **(Currently Amended)**      The method of Claim 1 Claim 10 wherein the DC are from peripheral blood.

12.      **(Currently Amended)**      The method of Claim 10 wherein the DC express

one or more <u>molecules selected from the group consisting</u> of MHC Class I molecules, MHC Class II molecules, CDT, CD4, CDllc, CD123, CD8a, CD205, 33D1, CD40, CD80, CD86, CD83, CD45, CMRF-44, CMRF-56, CD-209, CD208, CD207 ~~and/or~~ and CD206.

13.　　**(Currently Amended)**　　The method of Claim 1 wherein the antigen is <u>selected from the group consisting of:</u> a peptide, polypeptide, protein, nucleic <u>acid</u> ~~and~~ molecule, carbohydrate molecule, organic <u>molecule, and</u> ~~or~~ inorganic molecule.

14.　　**(Original)**　　The method of Claim 11 wherein the antigen is a peptide.

15.　　**(Currently Amended)** A method for priming T-cells *in vitro* for a target antigen, said method comprising<u>:</u>

~~co-incubated~~ <u>co-incubating</u> together or at different times, mature activated DC, CD4+ T-cells and CD8+ T-cells in the presence of said target antigen for a time and under conditions sufficient for CD8+ cytotoxic T-cells to generate with specificity for said antigen<u>;</u> and

~~then~~ isolating said CD8+ T-cells.

16.　　**(Original)**　　The method of Claim 15 wherein primed T-cells are generated in from about three to about 20 days.

17.　　**(Currently Amended)**　　A method of treatment of a subject comprising<u>:</u>

~~first~~ identifying a target antigen by screening for primed T-cells reactive to said antigen by the method of co-incubating mature, activated DC, CD4+ /CD25- T-cell and CD8+ T-cells in the presence of said target antigen for a time and under conditions sufficient for CD8+ cytotoxic T-cells to generate with specificity for said antigen<u>;</u> ~~and~~

~~then~~ isolating said CD8+ T- cells<u>;</u> ~~and~~

~~then~~ generating a vaccine based on an antigen to which T-cells are capable of being primed *in vitro*<u>; and</u>

<u>administering said vaccine to said subject in an amount effective to treat said subject.</u>

18.　　**(Canceled)**

19.　　**(Canceled)**

20.　　**(Currently Amended)**The method of Claim 17 ~~or 18 or 19~~ wherein the subject in a human, livestock animal, laboratory test animal, a captured wild animal or an avian species.

21.　　**(Original)**The method of Claim 20 wherein the subject is a human.

22. **(Currently Amended)**The method of <u>any one of Claims</u> ~~Claim 1 or~~ 17, <u>29 or 30,</u> ~~for the treatment of~~ <u>wherein the subject is treated for a condition selected from the group</u> <u>consisting of</u> hepatitis type A, hepatitis type B. hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I (HSV-I), herpes simplex type II (HSV-II) , rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, hantavirus, coxsackie virus, mumps virus, measles virus, rubella virus, polio virus, human immunodeficiency virus type I (HIV-I) and human immunodeficiency virus type II (HIV- II).

23. **(Currently Amended)**The method of <u>any one of Claims</u> ~~Claim 1 or~~ 17, <u>29 or 30,</u> ~~for the treatment of~~ <u>wherein the subject is treated for an infection selected from the group</u> <u>consisting</u> of ~~infection by~~ Mycobacterium, Rickettsia, Mycoplasma, Neisseria and Legionella.

24. **(Currently Amended)** The method of <u>any one of Claims</u> ~~Claim 1 or~~ 17, <u>29 or 30,</u> ~~for the treatment of~~ <u>wherein the subject is treated for an infection selected from the group</u> <u>consisting</u> of ~~infection by~~ Leishmania, Coccidioidomycoses and Trypanosoma.

25. **(Currently Amended)**The method of <u>any one of Claims</u> ~~Claim 1 or~~ 17, <u>29 or 30,</u> ~~for the treatment of~~ <u>wherein the subject is treated for an infection selected from the group</u> <u>consisting</u> of ~~infection by~~ Chlamydia and Rickettsia.

26. **(Currently Amended)**The method of <u>any one of Claims</u> ~~Claim 1 or~~ 17, <u>29 or 30,</u> ~~for the treatment of~~ <u>wherein the subject is treated for a condition selected from the group</u> <u>consisting</u> of fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangio-endotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcimona, basal cell carcinoma, adenocarcinoma, seat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, paillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma,

meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, e.g. acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia and heavy chain disease.

27.     **(Canceled)**

28.     **(Currently Amended)**      A pharmaceutical composition comprising the primed T-cells of ~~Claim 28~~ Claim 1 or 15.

29.     **(New)** A method of treatment of a subject comprising:

identifying a target antigen by screening for primed T-cells reactive to said antigen by the method of co-incubating mature, activated DC, CD4+ /CD25- T-cell and CD8+ T-cells in the presence of said target antigen for a time and under conditions sufficient for CD8+ cytotoxic T-cells to generate with specificity for said antigen;

isolating said CD8+ T- cells;

cloning and expanding the in vitro primed cytotoxic T-cells; and

administering said T-cells to a subject.

30.     **(New)** A method of treatment of a subject comprising:

identifying a target antigen by screening for primed T-cells reactive to said antigen by the method of co-incubating mature, activated DC, CD4+ /CD25- T-cell and CD8+ T-cells in the presence of said target antigen for a time and under conditions sufficient for CD8+ cytotoxic T-cells to generate with specificity for said antigen;

isolating DC/CD4+ T-cells primed for a particular antigen; and

administering said DC/CD4+ T-cells in an amount effective to treat said subject.